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Phytochemical composition and antibacterial properties of the essential oil of *Achillea biebersteinii* Afan. (Asteraceae)

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ABSTRACT

Background and aims: The aim of this study was to characterize the chemical composition and antimicrobial properties of *Achillea biebersteinii* essential oil (EO).

Methods: The chemical composition of samples obtained from Marand city in East Azerbaijan, was assessed using gas chromatography mass spectrometry (GC/MS). The antimicrobial properties were evaluated by the disc diffusion method against methicillin-resistant *Staphylococcus aureus* (MRSA), other extended-spectrum beta-lactamases (ESBLs) producing, as well as Gram-negative and Gram-positive bacteria. The minimum inhibitory concentration (MIC) value of EO was assessed using the agar dilution method.

Results: In *A. biebersteinii* the major compounds were α-terpinene (41.42%), 2-carene (13.96%), m-cymene (13.41%) and 1,8-cineole(8.91%).The EO showed antimicrobial activity against ten microorganisms, especially *Streptococcus sanguis*, *S. aureus* (MRSA strain), and *Klebsiella pneumoniae* (ESBL-producing strain), which was potentially better than tetracycline and kanamycin.

Conclusion: This study confirmed that EO of *A. biebersteinii* has *in vitro* antimicrobial activity against Gram-negative and Gram-positive bacteria, which has made it an alternative antibacterial agent.

Keywords: Biological activity, Cultivation, Essential oil composition, Thymus fedtschenkoi

Original article

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INTRODUCTION

Staphylococcus aureus is of great concern in healthcare and community settings, due to involvement in life-threatening infections, and development of resistance to most classes of antimicrobial agents. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a cause of healthcare associated infections, which had a dramatic increase in number in the 1990s [1], and the recent emergence of MRSA in community-associated infections highlights the success of this species as a pathogen and its ability to adapt under pressure from antimicrobial agents [2-4]. On the other hand, different reports on extended spectrum β-lactamases (ESBLs) variants and Metallo-beta-lactamases in the second half of the 1980s and broad geographical distribution of bacteria producing these enzymes, have been considered as another epidemiological phenomenon [5]. Carbapenems are often the last treatment option against ESBL-producing organisms. These organisms have become increasingly resistant to quinolones, aminoglycosides, trimethoprim-sulfamethoxazole and other antibiotics. Continuous consumption of carbapenems has resulted in the emergence of new classes of Gram-negative bacteria, which is known as superbugs [6]. Multidrug-resistant bacteria have an excessive life-threatening importance, not only in the USA, Europe and Japan but also in undeveloped countries [7], which have prompted researchers to think of new drugs.

Nowadays, due to these problems, new antimicrobial agents and medicinal plants are being investigated as alternatives, especially in countries with the preferred use of these types of drugs, such as Iran. Essential oils (EOs) of medicinal plants contain very potent natural biologically active agents [8]. The genus Achillea of Compositae (Asteraceae) contains about 150 species. It vegetates mostly on the territory of Europe and Western Asia as well as in Australia, New Zealand, and North America. *Achillea biebersteinii* is an annual plant that grows in Asia, and mostly in Europe, and is cultivated in Pakistan, and Iran, which made it as a target of medicinal plants. Geographic distribution of these species in Iran is in Azerbaijan, Isfahan, Fars, Kerman, Sistan and Baluchestan, Khorasan, and Tehran provinces [9]. These provinces have different ecology, which affects the efficiency of plants. Studies by Khosravi *et al.* [10] and Hassanshahian *et al* [11]. Showed antifungal and antimicrobial activities of this medicinal plant against clinical isolates, respectively. Thus, the present study was designed to assess antibacterial activity of EO of *A. biebersteinii* collected from East Azerbaijan province of Iran on Gram-negative and Gram-positive standard strains.

METHODS

Plant materials. Aire parts of *A. biebersteinii* were collected in Marand

city, East Azerbaijan. The collected materials were air-dried at room temperature (25°C) in the shade and powdered using a laboratory blender. The studied sample was confirmed as *A. biebersteinii* by a botanist.

Essential oil extraction. One hundred grams of each sample was mixed with 500 ml of distilled water and subjected to hydrodistillation using a Clevenger-type apparatus for 3 h until total recovery of oil. The preparation of the EO was performed three times, and the obtained oils were dehydration over sodium sulfate, weighed, and stored at 4°C until use.

Gas-chromatography mass spectrometry (GC/MS). The obtained EOs were analyzed using an Agilent 6890 gas chromatograph-mass spectrometer (GC/MS) fitted with HP- 5MS capillary column (30 m×0.25 mm) coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, Canada). The compounds were identified by matching their recorded mass spectra with the data bank mass spectra (Wiley 7N library).

Strains and growth culture. The antibacterial activity tests included 5 Gram-positive and 5 Gram-negative bacteria acquired from the American Type Culture Collection (ATCC) and the Persian Type Culture Collection (PTCC) including *Enterococcus faecalis* (ATCC 29212), *S. aureus* (ATCC25952), *S. aureus* (ATCC33591) as MRSA, *S. aureus* (ATCC29213), *S. sanguis* (PTCC1449), *Enterobacter aerogenes* (ATCC13048), *Klebsiella pneumoniae* (ATCC700603) as an extended spectrum β-lactamases producing bacteria, *Proteus mirabilis*(ATCC43071), and *Escherichia coli* O157:H7 (purchased from Razi Institute of Iran). These strains were kept at -70°C in Trypticase Soy Broth (TSB) with 20% glycerol, inoculated in Blood Agar (BA), and incubated overnight at 35°C. Subsequently, one colony from each culture was inoculated into TSB and incubated at 35°C for 24 h with shaking (100 rpm) in order to obtain freshly microbial culture suspension (108 CFU/ml) for tests.

Agar disc diffusion method. The antimicrobial activity of EOs was determined with the disc diffusion method according to Clinical and Laboratory Standards Institute guidelines [12]. Briefly, bacterial suspensions (108 CFU/ml) were spread on Mueller-Hinton Agar (MHA) using sterile cotton swabs. Then, filter paper discs (6 mmØ; Mast, UK) were impregnated with 10 µl of essential oils of *A. biebersteinii* and were placed on the surface of Petri dishes. Tetracycline (30 µg/disc) and Kanamycin (30 µg/disc) (Himedia, India), were used as the control. The tests were performed in duplicate for each strain. Antibacterial activity was evaluated by measuring the radius of the inhibition zone to the nearest millimeter.

Determination of minimum inhibitory concentration (MIC). The MIC of *A. biebersteinii* EO was determined usingagar dilution method [13]. The twofold serial broth dilution of EO was prepared and delivered to series of MHA plates at 45°C, resulted in a final concentration of 512 µg/ml to 0.5 µg/ml of essential oils. A standardized suspension of

studied bacteria (106 CFU) was inoculated into each plate (12 plates for each series), including the plate without EO as a growth control. The plates were incubated at 35°C for 24 h. The microorganisms that were sensitive to EO in the agar plates didn't grow at the inoculation site, where those that were resistant appeared as circular colonies.

RESULTS

The chemical composition of the essential oil. Hydrodistillation of the A. *biebersteinii* yielded EO. Their GC and GC/MS analysis led to the identification and quantification of 15 components (Table 1), representing 93.79% of the total oil. The oil was dominated by Monoterpenoids (90.63%) and Sesquiterpenoids (2.15%). The major components of the EO were α- Terpinene, 2- Carene, and m- Cymene (41.42, 13.96, and 13.41%), respectively.

Disc diffusion agar and agar dilution methods. In the screening of antibacterial activity of *A. biebersteinii* EO by disc diffusion method, the greatest inhibition zone (50±3 mm) was against *Streptococcus sanguis* PTCC

Table1. Chemical composition of *A.biebersteinii* collected from East Azerbaijan, Iran

P.N	Name	RI[a]	Area%
1	α- Pinene	938	**0.77**
2	Camphene	950	**0.96**
3	β- Pinene	977	**0.51**
4	2- Carene	996	**13.96**
5	α- Terpinene	1008	**41.42**
6	m- Cymene	1013	**13.41**
7	1, 8- cineol	1026	**8.91**
8	Terpinene	1052	**0.86**
9	Linalo	1099	**0.98**
10	2- Cyclohexen- 1 a	1123	**1.01**
11	Camphor	1148	**3.62**
12	α- Terpineol	1180	**3.18**
13	Piperitone	1228	**0.96**
14	Germacrene D	1479	**2.19**
	Total identified		**93.79**
	Monoterpenoids		**90.63**
	Sesquiterpenoids		**2.15**
	others		**1.01**

Retention indices measured for n-alkanes (c-9 to c-24) on the nonpolar DB-5 column.

Table 2. Results of MICs and agar disc diffusion tests for tested bacteria against EO of *A.biebersteinii*

Test microorganisms	Disk diffusion agar (mm)			MIC (µg/ml)
	EO	TE	K	EO
Enterococcus faecalis ATCC 29212	33±0.5	26±0.5	25±3	32
Staphylococcus aureus ATCC 25952	45±2	20±1	20±1	4
Streptococcus sanguis PTCC 1449	50±3	30±0.5	16±0.5	16
Staphylococcus aureus ATCC 33591	30±0.5	30±0.5	14±3	8
Staphylococcus aureus ATCC 29213	48±1	20±1	16±1	4
Enterobacter aerogenes ATCC13048	34±0.5	26±0.5	20±1	32
Klebsiella pneumoniae ATCC 700603	34±2	16±1	16±2	128
Escherichia coli ATCC25922	25±2	23±2	17±2	32
Proteus mirabilis ATCC43071	42±1	15±2	22±1	32
Escherichia coli O157:H7	40±3	22±0.5	20±0.5	32

EO: Essential oil; TE: Tetracycline; K: Kanamycin

1449, and the lowest MICs were for *S. aureus* ATCC 25952 and *S. aureus* ATCC 29213 (both 4 µg/ml, respectively). The results of the antimicrobial assays of EOs of A. *biebersteinii* are summarized in table 2. According to the results mentioned above, the growth of all tested microorganisms was inhibited by EO of *A. biebersteinii.* Figure 1 shows the results of the tested microorganisms' growth in plates containing serial dilutions of EO in 11 Petri dishes (512-0.05 µg/ml) as well as one plate without EO as a growth control.

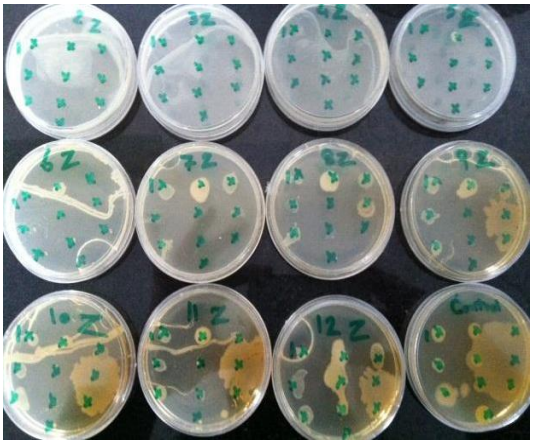

Fig. 1. MIC (0.5-512 µg/ml) of EO of T. copticum's seeds by Agar dilution method. 2z-12z, one series of diluted EO in agar; control, growth control of diluted microorganisms

DISCUSSION

The amounts of various compounds of EO of *A. biebersteinii* showed differences in samples taken from different part of Iran. In a study by Mahboubi *et al.* (2011), the major components of samples from Kashan city, Iran, were reported to be α-terpinene, and m-cymene (20.6, and 19%, respectively) [14]. Akbarnia *et al.* (2005) studied 12 samples from

different part of Qazvin, Iran, and found that the major components were α-terpinene and m-cymene (32.23 and, 16-25%, respectively) [15]. In another study by Haghiroalsadat *et al.* (2011) on A. *biebersteinii* harvested in Yazd province, Iran, it was revealed that α-terpinene (42.1%) were the most dominant components of the EO [16]. Rabiey *et al.* (2014), studied on A. *biebersteinii* harvested in Mashad, Iran, and reported that the most dominant components were m-cymene (22.55%), and α-terpinene (13.67%) [19]. A glance at the results, shows that the α-terpinene quantity of EO of *A. biebersteinii* obtained from Mashad city [17] was more than those of our study and Yazd, Qazvin, and Kashan [16-18], and α-terpinene quantity of our study was the same as that of the samples of Mahboubi *et al.*'s study (2011) in Kashan [14]. Based on the results of the antibacterial effect of EO of *A. biebersteinii,* our hypothesis was documented by growth inhibition zone varied from 25 to 50 mm in the disc diffusion method, and MIC varied from 4 to 32 µg/ml against the studied bacteria in agar dilution method. In a study by Shrivatara *et al.* (2012), on antimicrobial potential of Ajwain collected from India, it was shown that inhibition zone for *E. coli* MTCC-443, *Bacillus subtilis* MTCC-441, and *S. aureus* MTCC-3160 were 14.8, 13.6, and 9.9 mm, respectively [18]. Aggarwal *et al.* (2012) studied in Dehradun on 4 species of bacteria obtained from Culture Collection Center, National Culture Laboratory, Pune, India and found that inhibition zone of the oil for *Salmonella typhi*, *E. coli*, *Lactobacillus,* and *Bacillus licheniformis* were 40.45, 37.12, 44.54, and 0 mm, respectively [19]. A study by Oroojalian *et al.* (2010) on EO of *A. biebersteinii* showed similar MIC results for *E. coli* O157:H7 and *S. aureus* ATCC 25923 (6 and 25 µg/ml, respectively), in comparison with those of our study (32 and 4 µg/ml, respectively) [20]. The EO of *A. biebersteinii* collected from East Azerbaijan showed the same antibacterial activity as the other parts of Iran. Its antimicrobial activity was similar in MRSA strains to other strains of *S. aureus,* which indicated the importance of this medicinal plant as a new therapeutic agent. The antimicrobial activity of this essential oil against ESBLs-producing bacteria was less than MRSA strains and showed that the most activity was against *Streptococcus sanguis* (normal flora of the mouth), which is involved in dental caries. EOs leads to the destruction of microorganisms through the destruction of the cell wall and protein bacteria, interference with the action of membrane enzymes and transcription of DNA.

CONCLUSION

The α-terpinene (41.42%), 2-carene (13.96%) and m-cymene (13.41%) are significant compounds in the essential oil of *Achillea biebersteinii*. Also, the results of antibacterial activity of *Achillea biebersteinii* against *ten microorganisms, especially Streptococcus sanguis, S. aureus (MRSA strain), and*

Klebsiella pneumoniae (ESBL-producing strain) were shown this plant has good potential against *Streptococcus sanguis.* Based on this research and other reported this plant has a wide range of antibacterial properties and can be used in different industries such as pharmaceutical and food production.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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